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DATE MAILED: 01/19/2005

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/643,537	08/18/2003	Seok-Hwan Hwang	793-US	4268
75	90 . 01/19/2005		EXAM	INER
Albert Wai-Kit Chan		GELLNER, JEFFREY L		
Law Offices of	Albert Wai-Kit Chan,	LLC		
World Plaza, Suite 604		ART UNIT	PAPER NUMBER	
141-07 20th Av	enue .		3643	
Whitestone, NY	Y 11357		•	•

Please find below and/or attached an Office communication concerning this application or proceeding.

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		Application No.	Applicant(s)	
		10/643,537	HWANG ET AL.	
	Office Action Summary	Examiner	Art Unit	
		Jeffrey L. Gellner	3643	
Period fo	The MAILING DATE of this communication app or Reply	pears on the cover sheet with the c	orrespondence ad	Idress
A SH THE Exter fithe If NO Failu Any	ORTENED STATUTORY PERIOD FOR REPL' MAILING DATE OF THIS COMMUNICATION. nsions of time may be available under the provisions of 37 CFR 1.1 SIX (6) MONTHS from the mailing date of this communication. reperiod for reply specified above is less than thirty (30) days, a repl period for reply is specified above, the maximum statutory period or tre to reply within the set or extended period for reply will, by statute reply received by the Office later than three months after the mailing ed patent term adjustment. See 37 CFR 1.704(b).	36(a). In no event, however, may a reply be tin y within the statutory minimum of thirty (30) day will apply and will expire SIX (6) MONTHS from , cause the application to become ABANDONE	nely filed s will be considered timel the mailing date of this c D (35 U.S.C. § 133).	
Status			,	
1)🖂	Responsive to communication(s) filed on <u>01 N</u>	ovember 2004.		•
· —		action is non-final.		
3)□	Since this application is in condition for alloward closed in accordance with the practice under E			e merits is
Disposit	ion of Claims			
5)□ 6)⊠ 7)□	Claim(s) 1-20 is/are pending in the application 4a) Of the above claim(s) is/are withdraw Claim(s) is/are allowed. Claim(s) 1-20 is/are rejected. Claim(s) is/are objected to. Claim(s) are subject to restriction and/or	wn from consideration.		
Applicati	ion Papers			
10)	The specification is objected to by the Examine The drawing(s) filed on is/are: a) acc Applicant may not request that any objection to the Replacement drawing sheet(s) including the correct The oath or declaration is objected to by the Ex	epted or b) objected to by the drawing(s) be held in abeyance. Section is required if the drawing(s) is ob	e 37 CFR 1.85(a). jected to. See 37 C	• •
Priority (	under 35 U.S.C. § 119			
<ul> <li>12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).</li> <li>a) All b) Some * c) None of:</li> <li>1. Certified copies of the priority documents have been received.</li> <li>2. Certified copies of the priority documents have been received in Application No</li> <li>3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).</li> <li>* See the attached detailed Office action for a list of the certified copies not received.</li> </ul>				
2) Notice 3) Information	et(s) ce of References Cited (PTO-892) ce of Draftsperson's Patent Drawing Review (PTO-948) mation Disclosure Statement(s) (PTO-1449 or PTO/SB/08) or No(s)/Mail Date	4) Interview Summary Paper No(s)/Mail D 5) Notice of Informal F 6) Other: <u>See Continu</u>	ate Patent Application (PT)	O-152)

Continuation of Attachment(s) 6). Other: translations of JP62-278922 and JP5-219834.

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#### **DETAILED ACTION**

#### **Priority**

Receipt is acknowledged of papers submitted under 35 U.S.C. 119(a)-(d), which papers have been placed of record in the file.

# Claim Objections

Claims 7, 15, and 19 are objected to because of the following informalities:

In claim 7, line 9 of text, "a" should be --an--.

Claim 15 appears to be a duplicate of claim 2.

Claim 19 appears to be a duplicate of claim 5.

Appropriate correction is required.

# Claim Rejections - 35 USC §103

The following is a quotation of 35 U.S.C. §103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 2, 4, 5, 7, 8, 10, 11, and 13-20 are rejected under 35 U.S.C. §103(a) as being unpatentable over Keggins et al. (US 4,544,637) in view of JP62-278922.

As to claim 1, Keggins et al. discloses a method for treating whey (abstract) comprising the step of separating proteins (abstract) from a whey stock solution ("sweet whey" of abstract)

to make a medium ("mother liquid" or "liquid medium," for example, col. 3 lines 59-68, col. 5 lines 62-68, example 1 of col. 8), placing the solution in a reactor (from "inoculated and incubated" of col. 9 line 24), adjusting the medium to pH 3.8-6 (col. 9 lines 61-63) and growing an aerobic organism (Table 9 of col. 10). Not disclosed is the organism being mushroom mycelia and placing in a reactor at 25-32 C. JP62-278922, however, discloses using a whey medium in growing mycelia (abstract in English) and it is old and notoriously well known to grow mushrooms in a reactor at between 25 and 32 C (see for example, Femor (abstract only); Ibrahim et al. (abstract only) and Tang et al. (abstract) for growing mushrooms at temperatures between 25 and 32 C). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Keggins et al. by using with mushrooms as disclosed by JP62-278922 so as to use a medium which also uses discarded soybean lees so as recycle a waste product.

As to Claims 2 and 15, the limitations of Claim 1 are disclosed as described above. Not disclosed is the reactor set at 28.3 C and pH of 4.2. It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify the method of Keggins et al. as modified by JP62-278922 so as to optimize the reactor so as to achieve a particular growth rate with a particular mushroom species.

As to Claim 13, Keggins et al. as modified by JP62-278922 further disclose obtaining a whey stock solution (abstract) and adding appropriate amount of acid or base to precipitate the protein (abstract, col. 3 lines 32-42 of Keggins et al.).

As to Claim 14, Keggins et al. as modified by JP62-278922 further disclose inoculating the protein-free solution (from "inoculated and incubated" of col. 9 line 24)

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As to Claim 16, the limitations of Claim 1 are disclosed as described above. Not disclosed is removing 90% of the organic substances in the protein-free solution. However, the culture would removing more than 90% of the organic substances in the supernatant depending upon population growth length. It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify the method of Keggins et al. as modified by JP62-278922 by growing the mushrooms a length of time to deplete 90% of the organics so as to efficiently use the medium.

As to claim 4, Keggins et al. discloses a method of culturing and organism (abstract) comprising the step of separating proteins (abstract) from a whey stock solution ("sweet whey" of abstract) to make a medium (for example, example 1 of col. 8), adjusting the medium to pH 3.8-6 (col. 9 lines 61-3), placing the solution in a reactor as a medium and placing the organism in the medium (from "inoculated and incubated" of col. 9 line 24) and growing an aerobic organism (Table 9 of col. 10). Not disclosed is the organism being mushroom mycelia and placing in a reactor at 25-32 C. JP62-278922, however, discloses using a whey medium in growing mycelia (abstract in English) and it is old and notoriously well known to grow mushrooms in a reactor at between 25 and 32 C (see for example, Femor (abstract only); Ibrahim et al. (abstract only) and Tang et al. (abstract) for growing mushrooms at temperatures between 25 and 32 C). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Keggins et al. by using with mushrooms as disclosed by JP62-278922 so as to use a medium which also uses discarded soybean lees so as recycle a waste product.

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As to Claims 5 and 19, the limitations of Claim 4 are disclosed as described above. Not disclosed is the reactor set at 28.3 C and pH of 4.2. It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify the method of Keggins et al. as modified by JP62-278922 so as to optimize the reactor so as to achieve a particular growth rate with a particular mushroom species.

As to Claim 17, Keggins et al. as modified by JP62-278922 further disclose obtaining a whey stock solution (abstract) and adding appropriate amount of acid or base to precipitate the protein (abstract, col. 3 lines 32-42 of Keggins et al.).

As to Claim 18, Keggins et al. as modified by JP62-278922 further disclose inoculating the protein-free solution (from "inoculated and incubated" of col. 9 line 24)

As to Claim 20, the limitations of Claim 1 are disclosed as described above. Not disclosed is removing 90% of the organic substances in the protein-free solution. However, the culture would removing more than 90% of the organic substances in the supernatant depending upon population growth length. It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify the method of Keggins et al. as modified by JP62-278922 by growing the mushrooms a length of time to deplete 90% of the organics so as to efficiently use the medium.

As to claims 7 and 10, Keggins et al. discloses a method for treating whey (abstract) or culturing mushrooms comprising the step of obtaining whey stock solution (abstract); adding appropriate amount of acid or base to precipitate the protein (abstract, col. 3 lines 32-42); separating the proteins to obtain a supernatant ("mother liquid" or "liquid medium," for

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example, col. 3 lines 59-68, col. 5 lines 62-68, example 1 of col. 8; separation at col. 3 lines 45-50); pasteurizing the supernatant (col. 3 lines 65-68); placing the supernatant in a reactor (from "inoculated and incubated" of col. 9 line 24); selecting an appropriate organism for optimal growth rates in the supernatant (example 3 of col. 9 lines 15-55); placing the organism in the supernatant inside the reactor (from "inoculated and incubated" of col. 9 line 24); inoculating the supernatant (from "inoculated and incubated" of col. 9 line 24); and, aerobically culturing an organism in the reactor (Table 9 of col. 10) at a pH 3.8-6 (col. 9 lines 61-63) Not disclosed is the organism being mushroom mycelia; placing in a reactor at 25-32 C; and, removing more than 90% of the organic substances in the supernatant. JP62-278922, however, discloses using a whey medium in growing mycelia (abstract in English); it is old and notoriously well known to grow mushrooms in a reactor at between 25 and 32 C (see for example, Femor (abstract only); Ibrahim et al. (abstract only) and Tang et al. (abstract) for growing mushrooms at temperatures between 25 and 32 C) and, the culture would removing more than 90% of the organic substances in the supernatant depending upon population growth length. It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Keggins et al. by using with mushrooms as disclosed by JP62-278922 so as to use a medium which also uses discarded soybean lees so as recycle a waste product and to grow a length of time to deplete 90% of the organics so as to efficiently use the medium.

As to Claims 8 and 11, the limitations of Claim 7 and 10 are disclosed as described above. Not disclosed is the reactor set at 28.3 C and pH of 4.2. It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify the method of

Keggins et al. as modified by JP62-278922 so as to optimize the reactor so as to achieve a particular growth rate with a particular mushroom species.

Claims 3, 6, 9, and 12 are rejected under 35 U.S.C. §103(a) as being unpatentable over Keggins et al. (US 4,544,637) in view of JP62-278922 in further view of JP2000-201647.

As to Claim 3, the limitations of Claim 1 are disclosed as described above. Not disclosed is the mushroom being *G. lucidum*. JP2000-201647, however, discloses growing *G. lucidum* on a medium. It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify the method of Keggins et al. as modified by JP62-278922 by growing *G. lucidum* as disclosed by JP2000-201647 so as to have a useful foodstuff (see JP2000-201647 at abstract in English).

As to Claim 6, the limitations of Claim 4 are disclosed as described above. Not disclosed is the mushroom being *G. lucidum*. JP2000-201647, however, discloses growing *G. lucidum* on a medium. It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify the method of Keggins et al. as modified by JP62-278922 by growing *G. lucidum* as disclosed by JP2000-201647 so as to have a useful foodstuff (see JP2000-201647 at abstract in English).

As to Claim 9, the limitations of Claim 7 are disclosed as described above. Not disclosed is the mushroom being *G. lucidum*. JP2000-201647, however, discloses growing *G. lucidum* on a medium. It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify the method of Keggins et al. as modified by JP62-278922 by

growing *G. lucidum* as disclosed by JP2000-201647 so as to have a useful foodstuff (see JP2000-201647 at abstract in English).

As to Claim 12, the limitations of Claim 10 are disclosed as described above. Not disclosed is the mushroom being *G. lucidum*. JP2000-201647, however, discloses growing *G. lucidum* on a medium. It would have been obvious to one of ordinary skill in the art at the time of the invention to further modify the method of Keggins et al. as modified by JP62-278922 by growing *G. lucidum* as disclosed by JP2000-201647 so as to have a useful foodstuff (see JP2000-201647 at abstract in English).

#### Response to Arguments

Applicant's arguments filed 1 November 2004 have been fully considered but they are not persuasive. Applicant's arguments are: (1) Keggins et al. does not disclose or suggest that the optimum condition for culturing mushroom mycelia in whey is the temperature of 25 to 32 C with a pH of 3.8 to 4.6. (Remarks page 10, 1<sup>st</sup> complete para.); (2) JP62-278922 does not disclose or suggest that the optimum condition for culturing mushroom mycelia in whey is the temperature of 25 to 32 C with a pH of 3.8 to 4.6. (Remarks page 11, top incomplete para.); (3) Neither Keggins et al. nor JP62-278922, along or combined, teach removal and disposal of the organic substances (Remarks page 11, 2<sup>nd</sup> complete para.); (4) It is not old and notoriously well known to grown mushrooms in a reactor at between 25 and 32 C (Remarks page 11, last para.); (5) Keggins et al. does not disclose adjusting the medium to a pH of 3.8 to 4.6 because Keggins et al. raises the pH to 9 when precipitating the protein and only uses pH 4 to observe the

appearance of the medium (Remarks page 13 1<sup>st</sup> and 2<sup>nd</sup> para.); and, (6) the object of JP2000-201647 is different than the instant application (Remarks page 14, last para.).

As to arguments (1) and (2), Examiner agrees that Keggins et al. does not disclose an optimum condition for culturing mushroom mycelia in whey is the temperature of 25 to 32 C. Keggins et al. does disclose the use of a whey culturing medium at a pH of 3.8 to 4.6 (pH 4 or 4.5 at Table 8). JP62-278922 discloses culturing mycelia and it is know to use a temperature of 25 to 32 C to culture mushrooms (see for example, Femor (abstract only); Ibrahim et al. (abstract only) and Tang et al. (abstract) for growing mushrooms at temperatures between 25 and 32 C). With these references, Examiner considers it obvious to one of ordinary skill in the art to combine the references since all are in the general area of culturing of organisms.

As to argument (3), while none the references explicitly disclose the deposal of organic substances in the culturing medium, this result inherently follows when the medium is used over a length of time since the organism will use the organic substances during their growth.

As to argument (4), Examiner considers it old and notoriously well known to grow mushrooms in a reactor at between 25 and 32 C (see for example, Femor (abstract only); Ibrahim et al. (abstract only) and Tang et al. (abstract).

As to argument (5), although the embodiments of the Keggins et al. concentrate of media with greater pH values, it is settled law that "patents are relevant as prior art for all they contain" (see MPEP 2123 for citations). In Keggins et al. the value of pH 4 for the medium is disclosed and it is well known to adjust a culturing medium to the needed pH depending upon use.

As to augment (6) although JP2000-201647 has a different objective than the instant application, it is settled law that "patents are relevant as prior art for all they contain" (see MPEP 2123 for citations). JP2000-201647 discloses the culturing of G. lucidum.

#### Conclusion

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Jeffrey L. Gellner whose phone number is 703.305.0053. The Examiner can normally be reached Monday through Thursday from 8:30 am to 4:00 pm. The Examiner can also be reached on alternate Fridays.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's Supervisor, Peter Poon, can be reached at 703.308.2574. The official fax telephone number for the Technology Center where this application or proceeding is assigned is 703.872.9306.

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Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703.308.1113.

Jeffrey L. Gellner Primary Examiner



# **MACHINE-ASSISTED TRANSLATION (MAT):**

(19)【発行国】

(19)[ISSUING COUNTRY]

日本国特許庁(JP)

Japan Patent Office (JP)

(12)【公報種別】

(12)[GAZETTE CATEGORY]

公開特許公報(A)

Laid-open Kokai Patent (A)

(11)【公開番号】

(11)[KOKAI NUMBER]

特開平 5-219834

Unexamined Japanese Patent Heisei 5-219834

(43)【公開日】

(43)[DATE OF FIRST PUBLICATION]

平成5年(1993)8月31日

August 31, Heisei 5 (1993. 8.31)

(54)【発明の名称】

(54)[TITLE OF THE INVENTION]

104 E

ディスポーサブル培養袋及び培 Disposable culture bag and the culture method

養方法

(51)【国際特許分類第5版】

(51)[IPC INT. CL. 5]

A01G 1/04

A01G 1/04 104 E

【審査請求】 未請求

[REQUEST FOR EXAMINATION] No

【請求項の数】 3

[NUMBER OF CLAIMS] 3

【全頁数】 5

[NUMBER OF PAGES] 5

(21)【出願番号】

(21)[APPLICATION NUMBER]

特願平 4-59215

Japanese Patent Application Heisei 4-59215

(22)【出願日】

(22)[DATE OF FILING]

平成4年(1992)2月14日

February 14, Heisei 4 (1992. 2.14)

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【識別番号】

[ID CODE]

592057617

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(57)【要約】

(57)[ABSTRACT OF THE DISCLOSURE]

(Amendments Included)

【目的】

[PURPOSE]

(修正有)

温度調整された室内であれば In particular if it is chamber interior by which 特に除菌室を用いなくとも簡便 temperature control was carried out, even if it に、高価なジャーファーメンターと will not use microbe elimination chamber, it 同等の効果を奏し、且つ、ガラス shows effect easily equivalent to expensive jar のように割れるおそれもなく、融着 fermenter, and it can convey as it is only by



ーサブル培養袋を提供する。

するのみでそのまま輸送が可能 there being also no risk that it may be broken で、使用後は廃棄できるディスポ like glass, and fusing, and after usage provides destroyable disposable culture bag.

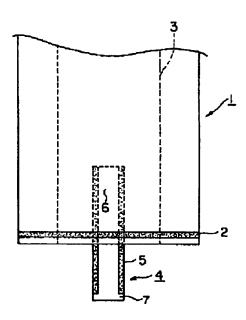
#### 【構成】

に当該培養袋に、液状ないし半 with said culture bag 培養方法。

# [CONSTITUTION]

培養袋底部にプラスチックフィ It inserts flat tube 4 which consists of plastic film ルムからなる扁平チューブ4を挿 into culture bag-bottom part, into culture bag 入し、該扁平チューブを内部貫通 which carried out fusion immobilization of this 部位6を閉塞することなく培養袋 flat tube at culture bag-bottom part without 底部に融着固定した培養袋並び blockading internal penetration part 6, together

流動性、或いは粉粒状の培養基 It is filled with liquid, semi-fluid, or particle-like を充填し、脱気孔を残して袋口を culture medium, it leaves vent and seals bag 密封し、袋口密封の前或いは後 opening, before bag-opening sealing or after, に、培地の滅菌を行い、袋底に設 the culture method of performing sterilization of けた扁平チューブから通気を行う medium and performing gas-passage from flat tube provided in bag bottom.





#### 【特許請求の範囲】

#### 【請求項1】

培養袋底部にプラスチックフィ に融着固定したことを特徴とする ディスポーサブル培養袋。

# 【請求項2】

項第1項記載のディスポーサブル by the above-mentioned. 培養袋。

#### 【請求項3】

ないし半流動性、或いは粉粒状 penetration part 或いは後に、培地の滅菌を行い、 方法。

#### 【発明の詳細な説明】

[0001]

[CLAIMS]

# [CLAIM 1]

It inserts flat tube which consists of plastic film ルムからなる扁平チューブを挿入 into culture bag-bottom part, it carried out fusion し、該扁平チューブを内部貫通部 immobilization of this flat tube at culture 位を閉塞することなく培養袋底部 bag-bottom part, without blockading internal penetration part.

> Disposable culture bag characterized by the above-mentioned.

#### [CLAIM 2]

扁平チューブに通気性フィルタ It provided air permeable filter in flat tube.

ーを設けたことを特徴とする請求 Disposable culture bag of Claim 1 characterized

#### [CLAIM 3]

培養袋底部にプラスチックフィ A culture method, which inserts flat tube which ルムからなる扁平チューブを挿入 consists of plastic film into culture bag-bottom し、該扁平チューブを内部貫通部 part, into culture bag which carried out fusion 位を閉塞することなく培養袋底部 immobilization of this flat tube at culture に融着固定した培養袋に、液状 bag-bottom part without blockading internal

の培養基を充填し、脱気孔を残し It is filled with liquid, semi-fluid, or particle-like て袋口を密封し、袋口密封の前 culture medium, it leaves vent and seals bag opening, before bag-opening sealing or after, it 袋底に設けた扁平チューブから performs sterilization of medium and performs 通気を行うことを特徴とする培養 gas-passage from flat tube provided in bag bottom.

#### [DETAILED DESCRIPTION OF THE INVENTION]

[0001]



#### 【産業上の利用分野】

送、取扱い容易なディスポーサブ 方法に関する。

#### [0002]

#### 【従来の技術】

一及び排気孔が最低限必要であ are need at least. などが設置されている。

#### [0003]

これら培養槽は小型のものは内 あり、通常ミニジャーファーメンタ level. ある。内容量の大きいものはジャ glass-made. に大型のものも使用されている。

#### [0004]

# [INDUSTRIAL APPLICATION]

本発明は、茸菌、各種バクテリア With this invention, while having effect which is などの通気培養に際し、ジャーフ equal to jar fermenter in the case of aeration ァーメンターに匹敵する効果を有 culture, such as mushroom mycelium and すると共に、軽量で簡便、且つ輸 various bacteria, it is lightweight and simple,

ル培養袋及びこれを用いた培養 It is an easily conveyed and handled and is an easy disposable culture bag, and it is related with the culture method using this.

#### [0002]

#### [PRIOR ART]

従来の培養槽はガラスまたはステ As for conventional culture tank, glass or シレス製の容器が使用され、その stainless steel vessel is used, sparger and 容器は通気のためのスパージャ exhaust hole of its container for gas-passage

り、その他撹拌機、サンプリングロ In addition, agitator, sampling mouth, etc. are installed.

#### [0003]

These culture tank

容量1リットルから5リットル程度で Small-sized things are 1 liter - 5 liter volume

ーと呼ばれ、主としてガラス製で It is usually called mini-jar fermenter, it is mainly

ーファーメンターと呼ばれ、主とし Large thing of net weight is called jar fermenter, てステンレス製で内容量10リット it is stainless steel and is mainly about 200 liter ルから200リットル程度であり、更 from 10 liter of net weight, furthermore, large sized thing is also used.

#### [0004]

小型のものは実験室レベルでの Many small-sized things to integration of data in データの集積や醗酵生産に多く laboratory level or fermentation production are 使用され、一度に数台ないし10 used, it may use several sets or about ten sets



数台を使用することもあるが、ー at once. 究者が利用できるのみであった。

般に高価であるため、限られた範 However, since it is generally expensiveness, it 囲の実験に使用され、一部の研 is used for experiment of limited range, it was only that some researchers can utilize.

#### [0005]

[0005]

# 【発明が解決しようとする課題】

近時、空調機器の普及及び効率 INVENTION] り、また、大型化されるに至った。 培養袋を設置することにより効率 高く、培養物の移動、輸送が容易 いた。

# [PROBLEM TO BE SOLVED BY THE

化により培養室などの温度制御が Recently, propagation and increase in efficiency 容易且つ精度高く行えるようにな of air-conditioning-machine device can perform temperature controls, such as culture このような室内に多数の同一系の room, easily and accurately, moreover, it came to extend.

よく培養できる環境が整うにした As environment which can be efficiently がい、簡易な設備で培養効率が cultivated by installing culture bag of many same types in such chamber interior is であり、使用後は廃棄できる培養 prepared, culture effectiveness is high at simple 容器及び培養方法が求められて installation, and movement of culture and transportation are easy.

> Discardable culture vessel after usage and culture method were sought after.

#### [0006]

# [0006]

#### 【課題解決の手段】

本発明は上記課題を解決するこ This

# [Means of business solution]

invention aims solving the at とを目的とし、その構成は、培養 above-mentioned problem, the configuration 袋底部にプラスチックフィルムから inserts flat tube which consists of plastic film なる扁平チューブを挿入し、上記 into culture bag-bottom part, into culture bag 該チューブを内部貫通部位を閉 which carried out fusion immobilization of the 塞することなく培養袋底部に融着 above-mentioned tube at culture bag-bottom 固定した培養袋に、液状ないし半 part without blockading internal penetration part 流動性、或いは粉粒状の培養基 It is filled with liquid, half-fluidity, or particle-like を充填し、脱気孔を残して袋口を culture medium, it leaves vent and seals bag 密封し、袋口密封の前或いは後 opening, before bag-opening sealing or after, it



ことを特徴とする。

# [0007]

は、内容物の重量に耐え、且つ、 であり、特にポリプロピレン、高密 度ポリエチレンなどのポリオレフィ ン系素材が好ましい。また、扁平 チューブの素材も培養袋素材と are desirable in particular. 同様の素材が使用できる。

#### [0008]

できる。中でもガセット袋は内容物 ましい形状である。

#### [0009]

培養袋の底部には扁平チューブ 着固定する。挿入にあたっては、

に、培地の滅菌を行い、袋底に設 is characterized by performing sterilization of けた扁平チューブから通気を行う medium and performing gas-passage from flat tube provided in bag bottom.

#### [0007]

本発明における培養袋の素材 Raw material of culture bag in this invention withstands weight of contents, and it is 125℃、30分以上の高圧滅菌に transparent plastic film which withstands 耐える透明なプラスチックフィルム degrees C and autoclaving for 30 minutes or more.

> Polyolefin-type raw materials. such as polypropylene and high density polyethylene,

> Moreover, raw material of flat tube can also use raw material similar to culture bag raw material.

# [8000]

本発明の培養袋の形状は特に限 Shape of culture bag of this invention does not 定はなく、チューブ状の袋素材の have limitation in particular, gusset bag which 両脇を折り込んだガセット袋、縦 inserted in both sides of tube-like bag raw 融着した筒状体或いはチューブ material, it folds up flatly cylindrical body or tube 状フィルムを扁平に折りたたみ、 like film which carried out vertical fusion, it can 横融着した袋、或いは縦融着した use bag which ligated end of bag which carried 筒状体或いはチューブ状フィルム out horizontal fusion, cylindrical body which の一端を結紮した袋などが使用 carried out vertical fusion, or tube like film.

Particularly, as for gusset bag, contents are を充填した場合に角形の袋底を stabilized in order to form square bag bottom, 形成するため内容物が安定し、好 when filled with contents, it is desirable shape.

#### [0009]

It inserts flat tube in bottom part of culture bag, it を挿入し、内部貫通部を残して扁 leaves internal penetration part and carries out 平チューブの両端を培養袋に融 fusion immobilization of the ends of flat tube at culture bag.

例えば、前もってフッソ樹脂やシリ In the insertion, for example, it draws out thin



残す方法がある。

コーン樹脂などの剥離性素材で plate, after performing bottom fusion or length 表面処理した薄板を挿入した扁 fusion at the same time it intercalates near bag 平チューブを、タテ融着部の袋底 bottom of length fusion part, and in bottom 付近や底融着部に挿入して扁平 fusion part flat tube which intercalated thin plate チューブを融着固定すると同時に beforehand surface-treated for removability raw 底融着或いはタテ融着を行った materials, such as fluorine resin and silicone 後薄板を引き抜き、内部貫通部を resin, and carries out fusion immobilization of the flat tube, there is method of leaving internal penetration part.

# [0010]

ムの両端を融着したものが一般的 common. であるが、細いフィルムを縦に二 However. 融着部と共に融着すれば使用で line part is fused with fusion part. た扁平チューブが好ましい。 給気 desirable. る。

#### [0011]

する。

[0012]

#### [0010]

本発明の培養袋の底部には扁平 It inserts flat tube in bottom part of culture bag チューブを挿入し給気孔を形成 of this invention, and forms air-supply hole.

する。 扁平チューブは細いフィル As for flat tube, what fused ends of thin film is

it double-folds thin film つ折りし、一方の端部が融着さ perpendicularly, one end part is fused, also that れ、他方の端部が折れ目線であ whose end part of another side is crease line, るもの、更には全く融着部を有し and cylindrical body which further completely ない筒状体も、折れ目線部位を does not have fusion part, it can use it, if crease

きる。しかしながら、両端を融着し However, flat tube which fused ends is

孔は袋内に少なくとも2cm、好ま Air-supply hole is at least 2 cm in bag, しくは3cm 以上の挿入部を設け preferably it provides insertion part 3 cm or more.

#### [0011]

給気孔の先端は閉塞せずに開口 It may be carrying out opening of the front end していてもよいが、先端を閉塞し of air-supply hole, without blockading.

て先端部近傍のフィルムに多数 However, if front end is blockaded and many の小孔を穿設すると、給気が微細 small holes are pierced on film near the leading 化して拡散し、給気効率が向上 end part, air supply will micronize and diffuse, trapping efficiency improves.

[0012]



ている部位の先端を融着せずに 残すことが好ましい。或いは表面 と裏面の長さを相違させたり、先 端の切り口を鋸歯状にするなどの 手段を講じることが好ましい。

給気孔の培養袋の外部に露出し Front end of part which it has exposed to ている部分の先端は、フィルム同 exterior of culture bag of air-supply hole, in 士が密着して開き難いため、扁平 order for films not to contact and open, it is チューブの培養袋の外に露出し desirable to leave without fusing front end of part which it has exposed to outside of culture bag of flat tube.

Or it lets the length of surface and back-side differ.

Moreover, it is desirable to provide means, such as to make cut end at front end into saw-tooth.

#### [0013]

法によって挿入固定すればよい。 菌注入孔としても使用できる。

#### [0013]

脱気孔としては、前もって培養袋 As vents, what is sufficient is just to carry out の上部に1個または2個以上の扁 insertion immobilization of the 1, or 2 or more 平チューブを給気孔と同様の方 flat tube by method similar to air-supply hole beforehand at upper part of culture bag.

その位置は培地を充填すべき位 The location should just be upper direction from 置より上方であればよい。この脱 location which should be filled with medium.

気孔はサンプリング孔、或いは種 It can use this vent also as sampling hole or a inoculum injected hole.

#### [0014]

によっても達成される。融着線の V1

#### [0014]

或いは脱気孔を設けず、袋口を Or it does not provide vent, when sealing bag 密封する際に脱気孔となるべき開 opening, it is attained also by leaving and 口部を残して不完全融着すること carrying out imperfect fusion of the vent which should constitute vent.

開口部は、脱気に支障なく同時 Vent of fusion line just eliminates encroachment に外部からの雑菌の侵入を排除 of various micro-organisms from outside できるものであればよい。或いは simultaneously convenient to deairing.

袋上部に穿孔を設け、この穿孔を Or it may provide perforation in bag upper part, フィルター素材で被覆してもよ and may cover this perforation with filter raw material.

# [0015]

#### [0015]

脱気孔、給気孔或いはその両者 It supplies air which will not be eliminated にウレタンフォームなど連続気泡 microbes if filter members, such as continuous



ことが好ましい。

# [0016]

する。例えば液体培地、流動性の distributed to whole. きる。更に、固形培地であっても medium, and semi-fluid medium. きる。

#### [0017]

を充填する。脱気孔として扁平チ 滅菌を行う。冷却後植菌を行う。 植菌は脱気孔を利用してもよく、 袋口を融着してもよい。

性プラスチック発泡体や繊維集合 cellular plastic foams, such as urethane foam, 体などのフィルター部材を挿入す and fiber assembly, are intercalated in vent. れば除菌されない空気を供給し air-supply hole, or both, it can prevent たり、除菌されない室内で培養し contamination, even if it cultivates in chamber ても汚染を防ぐことができる。フィ interior which is not eliminated microbes.

ルター部材は疎水性素材である As for filter member, it is desirable that it is hydrophobic raw material.

# [0016]

本発明に用いる培地は培養袋の Medium which it uses for this invention

底部から供給される空気が全体 It requires that it is raw material with which air に配分される素材であることを要 supplied from bottom part of culture bag is

培地、半流動性の培地も使用で For example, it can also use broth, fluid

粒状の素材であれば、各粒子と Furthermore, since spacing of each particle and 粒子の間隙空間が連続して存在 particles exists continuously if it is grain shape するため、この間隙を通過して供 raw material even if it is solid medium, it can 給空気を全体に配分することがで pass through this space and can distribute supply air to whole.

#### [0017]

本発明の培養袋を用いて培養す In order to cultivate using culture bag of this るには、培養袋に所定量の培地 invention, it fills culture bag with medium of predetermined amount.

ューブを培養袋上部に装着して When culture bag upper part is equipped with ある場合には、そのまま袋口を完 flat tube as a vent, it carries out full sealing of 全密封し、扁平チューブにパイプ the bag opening as it is, it inserts pipe in flat を挿入し、脱気孔を確保して加熱 tube, it secures vent and performs heat sterilization.

It performs inoculation after cooling.

また注射器状の器具を用いて行う Inoculation may utilize vent and it can also こともできる。或いは滅菌は袋口 perform it using syringe-like instrument.

を開いたまま行い、 植菌後に培養 Or it may perform sterilization, with bag opening opened, and it may fuse culture bag opening after inoculation.



# [0018]

れない開口部を残す。この場合、 ことが好ましい。開口部の上部に more preferably is desirable. に開口部から雑菌が侵入しがち sparse, である。

#### [0019]

同士の間隔は少なくとも1.5cm、 好ましくは2cm 以上離すことを要 preferably. 部を残して融着することも可能で inserted in. っても滅菌後に行ってもよいが、 滅菌前に行う場合には植菌は注 sterilization. 射器状の器具を用いて行う。

#### [0020]

#### 【作用】

#### [0018]

培養袋の上部に扁平チューブが When it is culture bag with which flat tube is not 設けられていない培養袋の場合 provided in upper part of culture bag, it leaves には、脱気孔として袋口に融着さ vent which is not fused by bag opening as a vent.

袋口融着部は袋口から少なくとも In this case, bag-opening fusion part is at least 2.5cm、好ましくは3cm 以上離す 2.5 cm from bag opening, separating 3 cm or

フィルム素材が少ないと、培養中 If film raw materials in upper part of vent is various micro-organisms tend to encroach from vent during culture.

#### [0019]

開口部を有する融着線を2本以 It provides two or more fusion lines which have 上設け開口部の位置をずらせるこ vent, and can also shift location of vent.

ともできる。この場合には融着線 In this case, intervals of fusion lines is at least 1.5 cm, it requires separating 2 cm or more

する。また、袋口を二重に折り込 Moreover, it inserts in bag opening doubly, and み、この折り込まれた部位を開口 it can leave vent and can also fuse this part

ある。袋口の融着は滅菌前に行 It may perform fusion of bag opening before sterilization, or it may carry out after

> However, when carrying out before sterilization, it performs inoculation using syringe-like instrument.

#### [0020]

#### [OPERATION]

本発明は培養槽として軽量なプラ This invention uses plastics bag lightweight as a スチック袋を使用し、給気用のノ culture tank, operation and transportation ズルとして同じくプラスチック製の became easy while there was effect which is 扁平チューブを培養袋に融着す equal to jar fermenter by putting on chamber



ーに匹敵する効果を奏すると共 from plastic into culture bag. に、操作、輸送が容易になった。 し、培地がこぼれるおそれがなく、 培養中には給気パイプを連結す air-supply pipe during culture. ることにより充分な空気を供給す ることができる。

ることにより、一定温度の室内に interior of constant temperature by fusing the 置くことにより、ジャーファーメンタ flat tube same as a nozzle for air supplies made

If flat tube is not opened according to external 扁平チューブは外力により開封し force, it blockades it with pressure of content ないと内容培地の圧力により閉塞 medium, there is no risk that medium may fall and it can supply sufficient air by connecting

#### [0021]

#### 【実施例】

ーブの袋本体との固定方法を示 す断面図、図3は他の実施例の 一部切欠を有する斜視図、図4は 視図である。

#### [0022]

1は培養袋、2は底融着部、3はガ セット折込み線である。底融着部 gusset insertion line. 2に扁平チューブ4を挿入固定し においては太線で表示した。5は at bottom fusion part 2. 2枚のフィルムを融着した融着部 であり、本実施例においては両側 thick line. に2本設け、中央部に内部貫通 5 is fusion part which fused film of two sheets. 残した非融着部である。

#### [0021]

#### [EXAMPLES]

図1は本発明の1実施例の袋底 FIG. 1 is top view showing bag-bottom part of 部を示す平面図、図2は扁平チュ one Example of this invention, FIG. 2 is sectional drawing showing fixing method with main body of bag of flat tube, FIG. 3 is a perspective diagram which has partially notch of 他の実施例の培養状態を示す斜 other Example, FIG. 4 is perspective diagram showing culture state of other Example.

#### [0022]

1 is culture bag, 2 is bottom fusion part, 3 is

It carried out intercalation immobilization of the 給気孔とした。底融着部2は、図2 flat tube 4, and considered it as air-supply hole

In FIG. 2, it displayed bottom fusion part 2 as

部位6を残した。7は給気孔の開 In this Example, it provides two at both sides, it 口を容易にするため融着せずに left internal penetration part 6 to center section. 7 is non-fusing part which it did not fuse, but was and left in order to make opening of air-supply hole easy.



# [0023]

図2における8は扁平チューブと 8 in FIG. 2 is fusion part of flat tube and bag film. 貫通部位6が融着されずに残っ た。図2においては内部貫通部位 6は閉塞しているが、内圧が加わ 6. れば容易に開口する。

#### [0024]

袋内部の先端9を閉塞した。 先端 inside culture bag. た。 給気孔である扁平チューブ4 front-end 9. ら微細な気泡となって上昇した。

#### [0025]

11は脱気孔であり、図1に示した 11 is vent. た。この場合には培地を充填し、

#### [0023]

袋フィルムとの融着部であり、扁 If flat tube 4 is inserted and bottom fusion is 平チューブ4を挿入して底融着を performed, film of 4 sheet will fuse mutually. 行えば4枚のフィルムが相互に融 However, since thin plate which provided Teflon 着するが、内部貫通部位6を設け film in part in which it should provide internal るべき部位にテフロン皮膜を設け penetration part 6 was intercalated and fusion た薄板を挿入して融着を行ったた was performed, after drawing out thin plate, it め、薄板を引き抜いた後は内部 remained, without fusing internal penetration part 6.

In FIG. 2, it blockades internal penetration part

However, if internal pressure is added, it will carry out opening easily.

#### [0024]

図3においては、培養袋1の底部 In FIG. 3, it provided fusion part 5 only in one に設けた扁平チューブ4の一方の side edge of flat tube 4 provided in bottom part 側縁にのみ融着部5を設け、培養 of culture bag 1, and blockaded front end 9

9近傍に多数の小孔10を穿設し It pierced many small holes 10 near the

から供給される空気は小孔10か Air supplied from flat tube 4 which is air-supply hole became fine bubble from small hole 10, and went up.

#### [0025]

扁平チューブとほぼ同様の部材 It fused flat tube shown in FIG. 1, and nearly をタテ融着部12の上方に融着し identical member above length fusion part 12. In this case, it is filled with medium, it fuses 上部を融着し、滅菌した後に脱気 upper part, after sterilizing, it vaccinates 孔11から菌を接種し、次いで扁 microbe from vent 11, subsequently, it 平チューブ4から給気を行い脱気 performed air supply from flat tube 4, and it was 孔11から脱気しつつ培養を行うこ able to perform culture, degassing from vent 11.



れることなくそのまま輸送すること without spilling. れば一層確実に輸送できる。

とができた。しかも培養後はこぼ And it was able to convey after culture as it was,

ができた。更に扁平チューブの外 Furthermore, if part which it has exposed to 部に露出している部位を融着す exterior of flat tube is fused, it can convey much more reliably.

# [0026]

袋の斜視図である。 扁平チューブ vent 11 in upper part. するための脱気孔を設ける必要 medium when sterilizing. 融着した。

#### [0026]

図4は上部に脱気孔11を設けな FIG. 4 is perspective diagram of FIG. 3 and い以外は図3とほぼ同様の培養 nearly identical culture bag except not providing

4から供給される空気を排出する In order to discharge air supplied from flat tube ため、或いは培地を充填後、滅菌 4, it is necessary to provide vent for discharging する際に発生する水蒸気を排出 water vapor generated after being filled with

がある。脱気孔として、袋口融着 As a vent, on the occasion of bag-opening の際に一部を開口部として残して fusion, it left part as a vent and fused it.

# [0027]

図4における13、14は融着線で 13 in FIG. 4 and 14 are fusion lines. けた。 融着線14から袋口までの vents 15 and 16. 内の空気は開口部15、2本の融 about 5 cm. れる。内圧が低下した場合には表 by culture bag exterior. 侵入を排除した。

# [0027]

あり、約2cm 間隔で2本設け、開 It provided two at intervals of about 2 cm, and 口部15、16をずらせた位置に設 provided in location which was able to shift

距離は約5cm であった。 培養袋 Distance from fusion line 14 to bag opening was

着線間の間隙17及び開口部16 Air in culture bag passes space 17 and vent 16 を通過して培養袋外部に排出さ of fusion line of 15 or 2 vents, and is discharged

裏の2枚のフィルム同士が密着し When internal pressure falls, films of two sheets て空気の通過を遮断し、雑菌の of front and back contact, and it interrupts passage of air, it eliminated encroachment of various micro-organisms.

#### [0028]

#### 実施例1

#### [0028]

#### Example 1

厚さ60 μ のポリプロピレン製で図 It injected medium of the following formula into



処方の培地を注入した。

ミキサー処理じゃがいも 60 micron.

400g

ル コ

60g

グ

イーストエキストラクト Malto extract

マルトエキストラクト PH 5.5

留

6g

蒸

1000ml

pH 5.5

[0029]

ウールを挿入して120℃で20分 vent 11, and sterilized for 20 minutes at 120 間殺菌した。発生する水蒸気は degrees C. 備培養しておいた舞茸の種菌20 vent 11. 4℃の室内にて底部に設けた扁 maitake 養した。

#### [0030]

ガ粉を用いる舞茸栽培用の種菌 as も、このまま輸送することが可能で circulation

3に示す形状の培養袋1に下記 culture bag 1 of shape shown in FIG 3 by product made from polypropylene of thickness

> Mixer treated potato 400g

60g ス Glucose

> Yeast extract 6g

6g

Distilled water 1000 ml

水

[0029] 袋口を融着し、脱気孔11にグラス It fuses bag opening, it inserted glass wool in

脱気孔11から破袋することなく順 Generated water vapor was discharged 調に排出された。冷却後、予め準 favorably, without carrying out bag tearing from

gを脱気孔11から接種し、室温2 It vaccinates after cooling 20g of inoculum of mushroom which carried 平チューブ4から除菌空気を約2 preliminary culture beforehand from vent 11, it 000ml/分の割合で供給して培 supplied and cultivated microbe elimination air at ratio of about 2000 ml/min from flat tube 4 provided in bottom part in chamber interior with a room temperature of 24 degrees C.

#### [0030]

7日間ないし10日間で菌糸の成 In seven days or in ten days make that the 長は一定となり、この培養物はオ growth of hypha is fixed, it has used this culture an inoculum for maitake-mushroom として使用可能となった。しかも、 cultivation which uses sawdust.

この種菌は輸送、流通に際して And this spawn conveys, in the case of



提供可能となった。

あり、液状種菌として茸栽培者に As it is, it can convey, as liquid inoculum, mushroom is provided for grower.

# [0031]

易であった。

## [0032]

# 実施例2

水分

広葉樹オガ粉 8リットル

フスマ

1リットル 65%

給して培養した。

#### [0033]

~550gの子実体が得られた。 degrees C. 収穫には通算45~75日を要す 30-35 days. る。

#### [0031]

通常、オガ粉培養種菌は接種後 Usually, sawdust culture inoculum needs 30日間の培養期間を必要とする incubation period for after vaccination 30 days. が、本発明の方法によれば7日な However, according to the method of this いし10日できわめて効率よく培養 invention, it can cultivate very efficiently in でき、しかもガラス製の容器と異な seven days or ten days, and, moreover, differs り、破損のおそれがなく輸送が容 from glass-made vessel, there was no fear of breakage and transportation was easy.

#### [0032]

Example 2

Broad-leaved-tree sawdust 8 liter

Wheat bran 1 liter

Water component 65%

上記処方の培地2.5kgを図4に示 It fills with the 2.5 kg above-mentioned medium す培養袋に充填し、図4に示すよ of formula culture bag shown in FIG. 4, as うに袋口を融着し、120℃で60分 shown in FIG. 4, it fuses bag opening, it 間殺菌した。冷却後、舞茸種菌1 sterilized for 60 minutes at 120 degrees C.

Ogを接種し、24℃の室内にて除 It vaccinates 10g of maitake-mushroom 菌空気を約50ml/分の割合で供 inoculum after cooling, it supplied and cultivated microbe elimination air at ratio of about 50 ml/min in 24-degree C chamber interior.

#### [0033]

約20日後、培地全体に菌糸が蔓 After about 20 day, after hypha spread in the 延した後、室温18℃の発生室に whole medium, it transfered to development 移動した。通算30~35日で400 chamber which is room temperature of 18

方、従来の方法によれば、舞茸の 400 - 550g fruiting body was obtained in total

On the other hand, according to conventional method, harvest of maitake mushroom takes total 45-75 days.



# [0034]

# 【発明の効果】

い。

#### 【図面の簡単な説明】

#### 【図1】

部を示す平面図である。

#### 【図2】

固定方法を示す断面図である。

#### 【図3】

有する斜視図である。

#### 【図4】

示す斜視図である。

#### 【符号の説明】

1 培養袋

#### [0034]

# [ADVANTAGE OF THE INVENTION]

本発明のディスポーサブル培養 In particular if it is chamber interior by which 袋を用いると、温度調整された室 temperature control was carried out when 内であれば特に除菌室を用いな disposable culture bag of this invention is used, くとも簡便な方法で、高価なジャ even if it will not use microbe elimination ーファーメンターと同等の効果を chamber, it can acquire effect equivalent to 得ることができる。しかも、割れる expensive jar fermenter by simple method.

おそれもなく、融着するのみでそ And there is also no risk that it may be broken, のまま輸送が可能であり、使用後 transportation is possible as it is only by fusing, は廃棄でき、流通上の利点も大き it can discard after usage and its advantage on circulation is also large.

#### [BRIEF DESCRIPTION OF THE DRAWINGS]

# [FIG. 1]

図1は本発明の1実施例の袋底 FIG. 1 is top view showing bag-bottom part of one Example of this invention.

# [FIG. 2]

図2は扁平チューブの袋本体との FIG. 2 is sectional drawing showing fixing method with main body of bag of flat tube.

#### [FIG. 3]

図3は他の実施例の一部切欠を FIG. 3 is a perspective diagram which has partially notch of other Example.

#### [FIG. 4]

図4は他の実施例の培養状態を FIG. 4 is perspective diagram showing culture state of other Example.

#### [DESCRIPTION OF SYMBOLS]

Culture bag

# JP5-219834-A



2 底融着部

3 ガセット折込み線

4 扁平チューブ

5 融着部

6 内部貫通部位

7 非融着部

8 扁平チューブと袋フィルムとの 8

融着部

9 先端

10 小孔

11 脱気孔

12 タテ融着部

13、14 融着線

15、16 開口部

17 間隙

2 Bottom fusion part

3 Gusset insertion line

4 Flat tube

5 Fusion part

6 Internal penetration part

7 Non-fusing part

8 Fusion part of flat tube and bag film

9 Front end

10 Small hole

11 Vent

12 Length fusion part

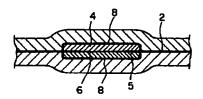
13 14 Fusion line

15 16 Vent

17 Space

【図2】

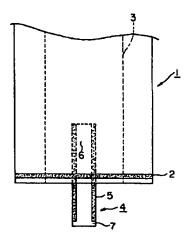
[FIG. 2]



【図1】

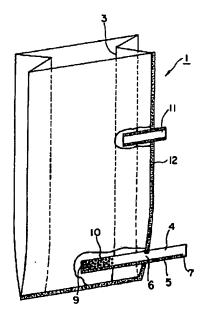
[FIG. 1]





【図3】

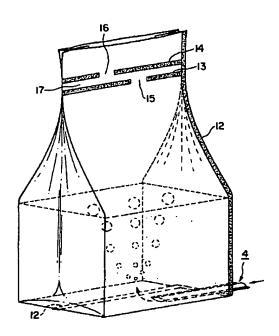
[FIG. 3]





【図4】

[FIG. 4]





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# MEDIUM FOR CULTIVATION OF EDIBLE MUSHROOMS

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UNITED STATES PATENT AND TRADEMARK OFFICE WASHINGTON, D.C. JULY 2004
TRANSLATED BY THE RALPH MCELROY TRANSLATION COMPANY

# JAPANESE PATENT OFFICE PATENT JOURNAL (A) KOKAI PATENT APPLICATION NO. SHO 62[1987]-278922

Int. Cl<sup>4</sup>: A 01 G 1/04

C 12 N 1/14

Sequence Nos. for Office Use: A-8502-2B

H-6712-4B

Filing No.: Sho 61[1986]-120599

Filing Date: May 26, 1986

Publication Date: December 3, 1987

No. of Claims: 1 (Total of 5 pages)

Examination Request: Not filed

#### MEDIUM FOR CULTIVATION OF EDIBLE MUSHROOMS

[Shokuyokinokono saibaiyo baichi]

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[There are no amendments in this patent]

#### Claims

- 1. Medium for cultivation of edible mushrooms, characterized in that the medium contains 40 wt% or more, based on total solids in the medium, dehydrated bean curd waste obtained by adjusting the bean curd waste pH to 3.5-5.5 and press dehydrating and adjusting to a pH suitable for cultivation of mushrooms.
- 2. Medium for cultivation of edible mushrooms according to Claim 1, wherein the pH adjustment of the bean curd waste is carried out by using an acidic solution obtained by lactic acid fermentation of a whey solution formed in the bean curd production process.

- 3. Medium for cultivation of edible mushrooms according to Claim 1, wherein the pH adjustment of the bean curd waste is carried out by using an acidic solution obtained by lactic acid fermentation of a whey solution formed in the milk product manufacturing process.
- 4. Medium for cultivation of edible mushrooms according to Claim 1, wherein the pH adjustment of the bean curd waste is carried out by using an acidic solution comprising an organic acid and/or an inorganic acid.
- 5. Medium for cultivation of edible mushrooms according to Claims 1 to 4, wherein the water content of the bean curd waste is 65-85 wt%.

# Detailed explanation of the invention

Application field of the invention

The present invention relates to a medium for cultivation of edible mushrooms.

#### Background of the invention

The method of utilizing a medium prepared by mixing sawdust and rice bran has been widely used for artificial cultivation of edible mushrooms until now.

In the conventional method utilizing the sawdust-rice bran medium, however, the following problems have been indicated. Namely, a large amount of sawdust to be mixed in the medium has been needed accompanied with active artificial cultivation on an industrial scale in recent years, but sawdust is generally obtained as a waste material from lumber and woodworking plants, etc. Therefore, the gathering and carrying of sawdust has become gradually more difficult as the demand has increased, leading to increase costs. Aside from this, in the case of cultivation using the aforementioned sawdust-rice bran mixed medium, the cultivation yield is low and the cultivation period becomes longer, and thus it production costs increase so that the advantage of artificial cultivation on an industrial scale is lost.

Commonly attempted solutions to the problems of effective utilization of waste from food plants have not related to the aforementioned matter. An example of these problems is the reutilization of bean curd waste (bean curd refuse) from the production of fresh bean curd, frozen bean curd, bean milk, etc. or the production of soy protein foods (separated soy protein, etc.). However, recently the reutilization of the aforementioned bean curd waste has faced a large problem because the demand as animal feed, a conventional utilization of for bean curd waste, has decreased and the quantity of supply from strained lees, etc. from the fermentation field for animal feed has increased. Further, the high water content of bean cured waste (typically 85%) raises transportation costs and makes its spoilage easy. Thus, in the summer of our country, it spoils after only 1 day, which is another problem for bean curd waste.

Thus, the utilization of bean curd waste as a medium for cultivation of edible mushrooms was considered in light of the aforementioned problems and the fact that bean curd waste has a relatively high content of nutrients used as medium ingredients, and the partial utilization of bean curd waste is actually being carried out. However, a large amount of chaff, etc. has to be added to adjust the water content to a suitable level in mediums for cultivation of edible mushrooms since the bean curd waste has a high water content as mentioned above; this lowers the utilization efficiency of nutrients in the bean curd waste and makes its advantage over the case of conventional sawdust-rice bran mixed mediums less attractive. Further, it has been considered to use the bean curd waste as the aforementioned medium ingredients after drying, but actual utilization has not occurred yet due to the high cost of drying.

#### Objective of the invention

The present invention was achieved in consideration of the aforementioned viewpoints, and its objective is to actually make possible the utilization of bean curd waste in mediums for cultivation of edible mushrooms as an effective use of bean curd waste and to realize efficient utilization of resources.

Further, another objective is to realize artificial cultivation of mushrooms at high yield by effectively utilizing the highly nutritive ingredients of bean curd waste.

#### Outline of the invention

The medium for cultivation of edible mushrooms of the present invention for realizing said objectives is characterized in that it contains 40 wt% or more, based on total solids in the medium, dehydrated bean curd waste obtained by adjusting the bean curd waste pH to 3.5-5.5 and press dehydrating and adjusting to a pH suitable for cultivation of mushrooms.

The edible mushrooms, for which the medium of the present invention is used, are not specifically restricted as long as they are appropriate for artificial cultivation, and the medium of the present invention is suitable especially for edible mushrooms such as agaricus, nameko, shiitake, etc.

As the bean curd waste in the present invention, that produced secondarily in the production of, for example, raw bean curd, frozen bean curd, bean milk, and the like or the production of soybean protein products (separated soybean protein, etc.) is utilized.

In the present invention, a press dehydration method, by which dehydration can be carried out at a low cost and high efficiency, is used for lowering the water content of the bean curd waste. However, the press dehydration is carried out after the bean curd waste is denatured by adjusting to pH 3.5-5.5 using an acidic solution since ordinary bean curd waste, as is, clogs the filter cloth, etc. of dehydration devices so that dehydration practically cannot be carried out.

The dehydration of bean curd waste is further improved if the pH is lowered to the range of 3.5-5.5. When the pH is below 3.5, a large amount of a neutralization agent is required in the subsequent pH adjustment to a medium for cultivation of edible mushrooms. Further, when the pH is higher than 5.5, the reduction of the water content by dehydration becomes insufficient or dehydration cannot be carried out.

As the acidic solution for pH adjustment of the aforementioned bean curd waste, organic acids such as lactic acid, citric acid, tartaric acid, malic acid, acetic acid, and the like, inorganic acids such as hydrochloric acid, phosphoric acid, and the like, or acidic solutions obtained by lactic acid fermentation of whey solutions produced in the bean curd production process or the production process of milk products (butter, cheese, etc.) are used, but when the acidic solutions obtained by lactic acid fermentation of whey solutions are used it is advantageous since the components in the whey solutions are utilized as nutrient ingredients of mediums for cultivation of edible mushrooms.

The aforementioned pH-adjusted bean curd waste is dehydrated by a press dehydration process utilizing a screw press, filter press, or the like to give dehydrated bean curd waste with a water content of about 60-80 wt%, preferably 65-75 wt%. The water content can be controlled by controlling the pressing force.

The dehydrated bean curd waste is adjusted to a pH suitable for cultivation of edible mushrooms and used as an edible mushroom medium directly or after adding necessary medium ingredients.

The adjustment to proper pH (generally pH 4.0-7.0) for cultivation of edible mushrooms is carried out by adding an alkali. The following alkalis can be used along or in combination of two or more. Namely, they are calcium carbonate, calcium hydroxide, calcium phosphate, calcium acetate, magnesium carbonate, etc.

In the present invention, the dehydrated bean curd waste can be used as is as a medium for edible mushrooms except for the pH adjustment, however suitable water content-controlling materials for controlling the water content of the medium may be added. As the water content-controlling materials, chaff is generally used for lowering the water content, and water is used for increasing the water content, but the water content-controlling materials are not limited to them.

One of special features of the medium for cultivation of edible mushrooms in the present invention is that the solids content in the dehydrated bean curd waste is 40 wt% or higher, preferably 50 wt% or higher, in terms of total solids in the medium even when suitable additives for adjustment of water content are added. When the solids content of the bean curd waste is less than 40 wt% of the total solids in the medium, the days cultivation of mushrooms cannot be

shortened and mushroom harvest is lowered so that the objective of the present invention cannot be sufficiently accomplished.

In the present invention, known additives for edible mushrooms may be used as mentioned above, and as such additives, for example, sawdust, rice straw, soybean husk, rice chaff, soy sauce cake, bean skins, starch, growth hormone, etc. can be exemplified.

The medium of the present invention can be used in the same way as conventionally mediums for cultivation of edible mushrooms. As an example, the medium containing the aforementioned dehydrated bean curd waste as a main component is put into a polypropylene container of a specified volume and sterilized in a sterilization kettle by wet heat after stoppering the container.

After cooling, the spores of edible mushroom are inoculated to the medium and cultivated at suitable temperature and other growth conditions.

#### Effect of the invention

According to the present invention, bean curd waste is used as the medium for cultivation of edible mushrooms so that effective utilization of resources, which could not be effectively utilized until now, can be realized.

Further, according to the present invention, artificial cultivation of edible mushrooms at a high yield can be realized by utilizing efficiently the highly nutritive ingredients of bean curd waste and an effect for making a great contribution to artificial cultivation of edible mushrooms on an industrial scale is obtained.

#### Application Examples of the invention

Next, the present invention is explained with Application Examples.

#### Application Example 1

#### Preparation of medium

# (1) Preparation of dehydrated bean curd waste:

An acidic solution, which was obtained by lactic acid fermentation of 25 L whey solution (waste solution) formed in the bean curd production process at 38°C for 12 h, was added to 5 kg, bean curd waste (water content 84.9 wt%), and the mixture was press dehydrated to obtain 3.4 kg dehydrated bean curd waste with a water content of 78 wt% and pH of 4.2.

#### (2) Adjustment of medium

The dehydrated bean curd waste obtained in (1) was mixed with 722 g chaff, 85 g, calcium carbonate and 275 mL water to prepare a medium with a water content of 67 wt% and a

bean curd waste solids content of 51 wt%, based on the total solids content of the medium, for cultivation of mushrooms.

#### Cultivation of edible mushroom

550 g of the aforementioned medium were placed in an 850-mL polypropylene container and sterilized by wet heat at 120°C for 1.5 h after stoppering the container tightly. Then, agaricus spores were inoculated and cultivated at a specified cultivation condition.

The results are shown in Table 1.

# Comparative Example 1

A medium was prepared in the same manner as that in Application Example 1 except that chaff was added to undehydrated bean curd waste to adjust the water content to 67 wt%, and agaricus was cultivated.

The results are shown in Table 1.

Table 1

	Cultivation days	Yield per bottle
Application Example 1	38-41 days	(average) 101 g
Comparative Example 1	51-53 days	(average) 87 g

As Table 1 shows, in the case of Application Example 1 of the present invention, the yield of young agaricus was increased as compared with Comparative Example 1, in addition the cultivation period was shortened, further the quality of the young agaricus produced was also superior.

# Application Example 2

#### (1) Preparation of dehydrated bean curd waste:

An acidic solution, which was obtained by lactic acid fermentation of 15 L whey solution (waste solution) formed in the bean crud production process at 40°C for 10 h, was added to 5 kg bean curd waste (water content 84.9 wt%), and the mixture was press dehydrated to obtain 3 kg dehydrated bean curd waste with water content of 75 wt% and pH of 4.2.

# (2) Adjustment of medium

The dehydrated bean curd waste obtained in (1) was mixed with 160 g pulverized rice straw and 85 g calcium carbonate to prepare a medium with a water content of 69.5 wt% and a

bean curd waste solids content of 76.7 wt%, based on the total solids content of the medium, for cultivation of edible mushrooms.

#### Cultivation of edible mushroom

In the same manner as that in Application Example 1, the medium was put in a container and sterilized by wet heat. After cultivating at 18-20°C and 70-75% relative humidity, spore were spread and cultivated in a growth chamber at 10-15°C and 90-95% relative humidity.

The results are shown in Table 2.

# Comparative Example 2

Water was added to 5 parts saw dust and 1 part rice bran to prepare a medium with a water content of 69.5 wt%, and agaricus was cultivated in the same manner as that in Application Example 2.

The results are shown in Table 2.

Table 2

	Cultivation days	Yield per bottle
Application Example 2	30-32 days	(average) 118 g
Comparative Example 2	52-56 days	(average) 78 g

As Table shows 2, in the case of Application Example 2 of the present invention, the yield of young agaricus was increased as compared with Comparative Example 2, in addition the cultivation period was shortened, further the quality of the young agaricus produced was also superior.

Further, in the case of Application Example 2, the development of hyphae was fast, and the developed hyphae were actively propagated to turn the medium purely white. In the case of Comparative Example 2, however, the development of hyphae was slow, and the developed hyphae made the medium faintly white.

# <u>Application Example 3</u>

# (1) Preparation of dehydrated bean curd waste:

An acidic solution (pH 4.6), which was obtained by lactic acid fermentation of 20 L whey solution (waste solution) formed in the cheese production process at 35°C for 4 h, was added 5 kg to bean curd waste (water content 84.9 wt%), and the mixture was press dehydrated to obtain 3.77 kg dehydrated bean curd waste with water content of 80 wt% and pH of 5.5.

# (2) Adjustment of medium

The dehydrated bean curd waste obtained in (1) was mixed with 500 g soybean husks and 333 g rice bran to prepare a medium with a water content of 67.1 wt% and a bean curd waste solids content of 50.0 wt%, based on the total solids content of the medium, for cultivation of edible mushrooms.

# Cultivation of edible mushroom

Agaricus was cultivated by the same manner as that in Application Example 2. The results are shown in Table 3.

# Comparative Example 3

Water was added to 6 parts saw dust and 1 part rice bran to prepare a medium with a water content of 67 wt%, and agaricus was cultivated in the same manner as that in Application Example 3.

The results are shown in Table 3.

Table 3

10010		
	Cultivation days	Yield per bottle
Application Example 3	34-36 days	(average) 113 g
Comparative Example 3	52-56 days	(average) 77 g

As it Table shows 3, in the case of Application Example 3 of the present invention, the yield of young agaricus was increased as compared with Comparative Example 3, in addition the cultivation period was shortened, further the quality of the young agaricus produced was also superior.

Further, in the case of Application Example 3, the development of hyphae was fast, and the developed hyphae actively propagated to turn the medium purely white. In the case of Comparative Example 3, however, the development of hyphae was slow, and the developed hyphae made the medium faintly white.

# Application Example 4

#### (1) Preparation of dehydrated bean curd waste:

An acidic solution containing 20 L 0.05% lactic acid was added to 5 kg bean curd waste (water content 84.9 wt%), and the mixture was press dehydrated to obtain 2.2 kg dehydrated bean curd waste with a water content of 66 wt% and pH of 3.6.

# (2) Adjustment of medium

The dehydrated bean curd waste obtained in (1) was mixed with 18 g calcium carbonate and 2 g calcium hydroxide and the pH was adjusted to prepare a medium with a water content of 65.4 wt% and a bean curd waste solids content of 97.4 wt%, based on the total solids content of the medium, for cultivation of edible mushrooms.

# Cultivation of edible mushroom

Agaricus was cultivated in the same manner as that in Application Example 2. Further cultivation of Comparative Example 4 was carried out using the medium of Comparative Example 2 as a comparison to Application Example 4.

The results are shown in Table 4.

Table 4

	Cultivation days	Yield per bottle
Application Example 3	34-36 days	(average) 113 g
Comparative Example 3	52-56 days	(average) 77 g

As Table 4 shows, in the case of Application Example 4 of the present invention, the yield of young agaricus was increased as compared with Comparative Example 4, in addition the cultivation period was shortened, further the quality of the young agaricus produced was also superior.

Further, in the case of Application Example 4, the development of hyphae was fast, and the developed hyphae actively propagated to turn the medium purely white. In the case of Comparative Example 4, however, the development of hyphae was slow, and the developed hyphae made the medium faintly white.